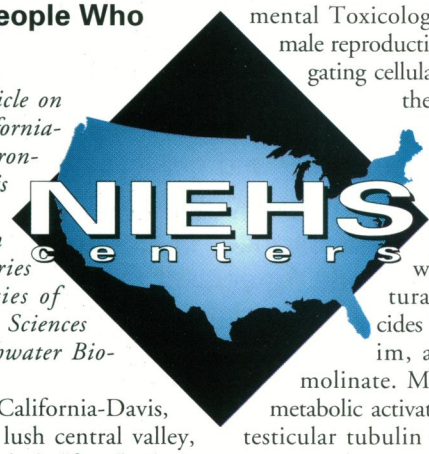
Protecting the People Who Feed Us



Editor's note: This article on the University of California-Davis Center for Environmental Health Sciences is the sixth in a series that appears intermittently in NIEHS News. The series highlights the activities of Environmental Health Sciences and Marine and Freshwater Biomedical Sciences Centers.

The University of California-Davis, located in California's lush central valley, began in 1905 as Berkeley's "farm" where students came to study agriculture. Since that time, UC-Davis has become a full-service campus. The UC-Davis Center for Environmental Health Sciences was established in 1992 and currently consists of 10 departments. Under the direction of Fumio Matsumura, a professor of environmental toxicology, the center takes an interdisciplinary approach to research in the fields of epidemiology, organ toxicity, biomarkers of exposure, and mechanistic toxicology.

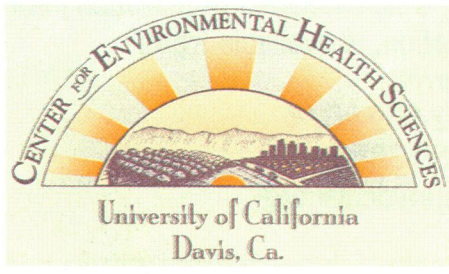
Studies of the occupational health of agricultural workers and the specific hazards associated with their work, such as dermal exposure to pesticides and inhalation of agricultural dusts, are conducted in collaboration with National Institute of Occupational Safety and Health and the State of California. Center investigators collect biomonitoring data in farmworker communities using blood and urine tests, chest radiograms, spirometry, and health and work questionnaires. In addition, soil is tested and air is monitored using portable air sampling pumps.

At the Institute of Toxicology and Environmental Health (ITEH), reproductive biologists Bill Lasley and James Overstreet focus on the reproductive health of farmworkers. Their goal is to develop assays for urinary hormone metabolites that can be used to detect reproductive toxicity in female or male workers. Tests are being developed that can detect infertility in women and men and menstrual dysfunction in women, as well as spontaneous abortion and abnormal pregnancy. Many of these assays have already been used by epidemiologists in studies of exposed workers.

Miller of the Department of Environmental Toxicology conducts research on male reproductive toxicity. She is investigating cellular mechanisms that make the testis uniquely vulnerable to adverse effects after exposure to environmental chemicals such as nitroaromatic compounds, many of which are used as agricultural chemicals, the fungicides benomyl and carbendazim, and the rice herbicide molinate. Miller studies the role of metabolic activation in testicular toxicity, testicular tubulin as a target for toxicant action, and immunocytochemical probes for assessing testicular damage. Her group has found that carbendazim, a metabolite of benomyl, inhibits testicular ß-tubulin assembly at a much lower concentration than that which affects the brain tubulins. The microtubules in the testis may have a role in these differential effects. Another study is focusing on the development of markers of toxic exposure in sperm. The study uses biophysical (motility, morphology), cellular, and biochemical approaches to develop markers for site-specific effects on spermatogenesis and/or sperm maturation.

Investigators in the animal/inhalation core are using cross-species studies to define possible effects of inhaling dust on farmworkers. Epidemiologic studies of California citrus and grape harvesters have shown an increased prevalence of signs of restrictive lung function compared to citrus orchard workers. Jerold Last and Hanspeter Witschi treated rats with single or repeated instillations of dust samples collected in the field. Responses of the lung were evaluated by analyzing lung lavage fluid, lung collagen content, histopathology, and cell kinetics. Dusts collected in vineyards have fibrogenic potential, whereas dusts collected in citrus groves are biologically less active. The animal/inhalation core will conduct more tests to confirm the effects of grape dust, assess the mechanistic basis of its ability to cause fibrotic lung changes, and identify the causative constituents.

There has been debate in recent years about the impact of rice straw on public health in the Sacramento Valley, the major rice-growing area of California. Much of the discussion has focused on amorphous silica fiberlike particles released by burning rice straw. There have been reports of unexplained cases of mesothelioma possibly associated with amorphous silica fiber exposure from sugar cane farming. Currently, the health effects of exposure to amorphous silica fibers are not known.



The ITEH has been working on a study of pulmonary pathology and mineral content of lung tissue from California farmworkers. Previous studies have suggested that California farmworkers have pneumoconiosis with inflammation and fibrosis associated with silica or silicate exposure and that restrictive pulmonary function may be associated with this population. The ITEH developed a protocol to evaluate subclinical histologic lesions and dust exposure. Histopathology of autopsy samples provides a method of systematically analyzing lung tissue for early, subclinical pneumoconioses in this population. Microdissection allows reproducible analysis of the lungs for histologic changes and morphometry.

Center investigators Dennis Hsieh and Daniel Jones are trying to determine the mutagenicity of fractions of combustion products of toxic wastes and diesel fuels. Mutagenicity of vehicular diesel exhausts, by-products from the incineration of plastics, and fumes of cooking meat were tested. About equal mutagenicity was found in the particulate and the vapor phases of the diesel exhausts. A toxic polyaromatic hydrocarbon from incineration of plastics was not mutagenic, and mutagenic heterocyclic amines were found in the aerosols of the cooking fumes but not in the vapor phase. Further studies will be performed to identify the major mutagenic components in vehicular diesel exhausts and the products of plastic incineration.

Jones is also characterizing the organic constituents of agricultural dusts using analytical techniques such as gas chromotography–mass spectrometry and liquid chromatography–mass spectrometry. Similar methods have been developed to detect pesticide metabolites excreted in urine such as mercapturic acids and dialkylphosphates. Electrospray ionization mass spectrometry has been used to determine the catalytic mechanism of epoxide hydrolase. Another class of important detoxifying enzymes, glutathione-*S*-transferases, consists of numerous individual isozymes whose distribution in tissues depends on gender. Mass spectrometry and enzyme assays are being used to investigate the mechanisms by which these enzymes become inactivated by exposure to reactive toxins.

A study led by Alan Buckpitt is looking at the metabolism and toxicity of a pesticide,

ethylene dibromide (EDB), in isolated Clara cells and in airway segments from mice and rhesus macaques. EDB is metabolized via cytochrome P450 monooxygenase-dependent pathways to bromoacetaldehyde (which is thought to be the reactive metabolite binding to protein). Earlier studies demonstrated substantial differences in P450-dependent metabolism at different airway levels and between species. The toxicity and tumorigenic effects of EDB may be site selective and this will depend on the balance of metabolic enzymes present in different airways. Although animal studies with EDB were quite conclusive, earlier epidemiology studies of lung diseases among workers exposed at relatively high levels were inconclusive. Center studies are intended to provide fundamental data on carefully defined subsections of the lung, leading to a better understanding of the metabolism of compounds like EDB.

The laboratory of Robert Rice in the Department of Environmental Toxicology studies the responses of human keratinocytes in culture as a model for epidermal toxicity. One focus is the growth inhibition and genotoxicity of mycotoxins and heterocyclic amines, which are greatly potentiated by inducers of cytochrome P450 activity such as TCDD. Rice and coworkers also study arsenic perturbation of differentiation that may contribute to its carcinogenicity.

Matsumura and Essam Enan have examined the effect of TCDD and other dioxin-type chemicals on glucose transport. They showed that TCDD reduced glucose-transport activity in normal keratinocytes in 1 hour at concentrations as low as 10 nanomolar. By contrast, TCDD stimulated glucose transport in immortalized keratinocytes. Further studies will address the molecular mechanism by which TCDD and its congeners alter glucose-transport activity, with emphasis on species and sex differences.

Some organophosphate esters cause long-term damage to the nervous system known as organophosphate-induced delayed neuropathy (OPIDN). The mechanism of toxicity underlying OPIDN is not known, but inhibition of an enzyme known as neuropathy target esterase (NTE) may be involved. Center investigators use highly differentiated nerve cultures, species sensitive to OPIDN such as the chicken, and protein isolation to study OPIDN and the role of NTE. Recent work showed specific effects of OPIDN-causing chemicals such as diisopropyl fluorophosphate excluded kinesin protein phosphorylation as a mechanism for OPIDN and demonstrated that phospholipase A_2 effectively solubilizes NTE in its active form. This work forms the basis for development of monoclonal antibodies to NTE.

UC-Davis CEHS

Agricultural workers are exposed to pesticides by a number of scenarios including foliar contact, dust from field preparation, aerial spraying, and burning.

Much of the work at the UC-Davis center focuses on the effects on target organs such as the brain, as well as the effects of xenobiotics at the cellular, molecular, and genetic levels. An example of a multilevel study is the research on epoxide hydrolase (EH). EH is found in most organs in the body and is believed to play a key role in the detoxification of oxides and epoxides. Such epoxides are, in many cases, the actual carcinogenic and mutagenic substances formed as the by-products of metabolism of environmental pollutants such as pesticides and polycyclic aromatic hydrocarbons. Charles Plopper has developed a method to locate distribution of EH in the kidney cells of the rat. Future studies will focus on EH in the lung and liver using light microscopy and ultimately electron microscopy to locate distribution of EH at the subcellular and suborganelle levels. David Grant and Bruce Hammock have also been working on several different EH projects including mapping of the soluble EH gene. Their goal is to determine how and to what extent EH affects differential susceptibility to toxic exposures.

Carboxylesterases are important in the metabolism of xenobiotics as targets of the action of several classes of toxins. In support of work on NTE, surrogate spectral substrates have been made that will be valuable in the purification of NTE. Spectral substrates for butyrylcholinesterase have been produced that are 10 times more sensitive than standard substrates. Similarly, new substrates for hepatic carboxylesterase assays have resulted in a 10–50 times increase in sensitivity.

Wilson is collaborating with Isaac Pessah on research into the maturation of acetylcholinesterase (AChE) forms in cultured quail myotubes. Ryanodine is a potent, naturally occurring plant toxin and insecticide.

Their studies have shown that persistent block of the sarcoplasmic reticulum calcium ion channel with ryanodine induces a transition from embryonic to mature forms of the enzyme. This is the first time maturation of AChE has been demonstrated in embryonic myotubes in culture, and it demonstrates an important role of calcium channels in the process. Center investigators are now researching ryanodine receptors in cultured human cells to study the effect of pesticides.

Pessah is also examining key calcium regulatory proteins within the microsomal membrane of muscle and nerve cells which appear to be the target of site-selective oxidation by various quinone structures. Quinonoid structures are ubiquitous in the environment, having both natural and anthropogenic sources. Human exposure to quinones can occur clinically (e.g., the antineoplastic anthraquinones) and by environmental exposure to diesel exhaust, cigarette smoke, and industrial particulate matter. Quinones are of significant concern to human health because their intrinsic electrophilicity can induce various patterns of acute and chronic oxidative damage to biological tissues. Quinone toxicity is closely associated with changes in cellular calcium regulation in a number of cell types. Ryanodine-sensitive calcium channels are uniquely sensitive to quinone-mediated oxidative insult. Recently, the center studies have shown that the microsomal membrane possesses a small number of highly nucleophilic thiols and that these thiols are located on the ryanodine receptor and its associated modulatory proteins. Inter- and intramolecular redox reactions between reactive sulfhydryls on the ryanodine receptor complex regulate calcium ion channel function and calcium transport across the microsomal membrane, which may be an important molecular basis for quinone-mediated toxicity. Pessah's laboratory is now determining the exact role that these altered macromolecules play in cellular dysfunction and organ-selective toxicity.

New Clues to Infant Mortality

Newborn babies in at least 20 countries have a better chance of surviving than those born in the United States. A paper in the March 1 issue of *The Journal of the American Medical Association* suggests that high rates of preterm delivery in the United States may be part of the problem.

For many years, researchers in the United States have focused on birth weight as a crucial component in infant mortality. Babies in Norway and other Scandinavian countries are heavier, and this was thought to be why more of them survived. "Our results suggest that birth weight itself is not the problem," said Allen Wilcox, the NIEHS epidemiologist who directed the study, conducted by an international team of researchers. Research comparing babies born in the United States and Norway showed that if the United States could lower its rates of preterm delivery to Norway's level, one of the lowest in the world, U.S. infant mortality might be decreased to Norway's level as well.

"The most surprising thing," said Wilcox, "is that even if we could reduce our preterm deliveries in the U.S. and lower our mortality, our birth weights would still be lighter than Norway's."

Wilcox points out that the study's findings are consistent with international trends. Japan and Singapore have surpassed the Scandinavian countries in achieving the lowest infant mortality rates in the world, despite the fact that babies weigh less in the Asian nations than in Scandinavia or the United States.

"We still don't know why the U.S. has more preterm deliveries than other countries," said Wilcox. "We don't even understand what causes labor to begin. But we do think these are the right questions to be asking. If we could aim more of our research towards figuring out how to prevent preterm deliveries, we might be able to make some real headway in lowering infant mortality."

The study was conducted as a collaboration among scientists at the National Institute of Environmental Health Sciences, the National Center for Health Statistics, the University of Bergen in Norway, and the Free University of Brussels in Belgium.